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Natural Reassortment of Eurasian Avian-Like Swine H1N1 and Avian H9N2 Influenza Viruses in Pigs, China

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Several zoonotic influenza A viruses detected in humans contain genes derived from avian H9N2 subtypes. We uncovered a Eurasian avian-like H1N1 swine influenza virus with polymerase basic 1 and matrix gene segments derived from the H9N2 subtype, suggesting that H9N2 viruses are infecting pigs and reassorting with swine influenza viruses in China.

Swine are regarded as a mixing vessel for influenza A viruses (IAVs) (1). Avian, swine, and human IAVs can co-infect pigs and generate novel reassortants of zoonotic or pandemic potential.

The emergence of pandemic H1N1 IAV (pH1N1), containing viral segments from avian, swine, and human viruses, highlighted the key role of pigs in contributing to IAV reassortment and evolution (2). Research in China also showed evidence of avian H5, H7, H9, and H10 influenza infections in pigs (3). Avian IAVs linked to human infection in this region contained internal genes derived from avian H9N2 viruses, indicating that the internal genes of the H9N2 virus might aid zoonotic transmission (4). We report detection of a swine IAV with polymerase basic (PB) 1 and matrix (M) gene segments of avian H9N2 origin.

In April 2021, we resumed monthly influenza surveillance program of imported pigs in a local slaughterhouse, which had been interrupted by COVID-19 outbreaks (5). We collected individual nasal swab samples (≈75 samples per visit), which we kept chilled in virus transport medium until they reached the laboratory. We then subjected swab samples to IAV isolation by using MDCK cells, as previously described (2). We identified cultures with cytopathic effect and tested them using a standard hemagglutination assay with turkey red blood cells. We tested hemagglutination-positive cultures with a universal influenza reverse transcription PCR assay specific for M segments (6). We studied samples that were positive for this reaction by using next-generation sequencing to deduce the full virus genomes (6).

During April 2021–February 2022, we collected a total of 829 porcine nasal swab samples (Table). We isolated 8 IAVs: 7 from August 2021 and 1 from September 2021. Virus sequences deduced from this study are available from GISAID (isolate nos. EPI_ISL_12471293–300). We compared those sequences with reference sequences (Appendix Table, <https://wwwnc.cdc.gov/EID/article/28/7/22-0642-App1.pdf>). IAVs detected in August 2021 were H3N2 viruses. The hemagglutinin (HA) and neuraminidase (NA) segments of those viruses were associated with human-like H3N2 swine influenza A virus; however, their internal gene segments all were derived from the pH1N1 lineage (Figure; Appendix Figures 1–6). These viruses were genetically not identical but highly similar. The influenza-positive pigs came from farms located in 2 provinces across southern China. Because this slaughterhouse followed a daily clearance policy requiring that all imported live pigs be slaughtered within 24 hours of admittance, our results suggest influenza transmission between pigs in the pre-slaughter transport chain outside Hong Kong. This

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Table. Swine influenza viruses detected in imported pigs, China, April 2021–February 2022

Year and month	No. nasal swabs	No. virus isolates	Isolation rate, %
2021			
Apr	60	0	0
May	75	0	0
Jun	75	0	0
Jul	75	0	0
Aug	75	7*	9.3
Sep	75	1†	1.3
Oct	79	0	0
Nov	85	0	0
Dec	80	0	0
2022			
Jan	75	0	0
Feb	75	0	0
Total	829	8	0.97

*All H3N2; pigs were imported from 2 provinces in southern China.

†H1N1; pig was from imported from a province in southern China.

H3N2 genotype was previously detected in pigs from Guangxi, China (7).

The swine H1N1 IAV that we isolated in September 2021, A/swine/HK/NS419/2021, a reassortant between multiple swine influenza lineages (Figure; Appendix Figures 1–6). The PB1 and M gene segments of this virus are of avian H9N2 virus subtype. This virus contains PB2, polymerase acidic, and NP gene segments derived from the pH1N1 lineage. Its HA and NA gene segments are of Eurasian avian-like H1N1 lineage, and its nonstructural gene segment is of a triple reassortant lineage. We further purified the isolated virus by using plaque assays to exclude the possibility of a mixed infection. We confirmed that all plaque-purified viral clones had an identical genotype.

The A/swine/HK/NS419/2021 isolate featured a PB1 gene segment of SH/F/98-like lineage and an M gene segment of G1-like H9N2 lineage (Figure). Similar PB1 and M sequences have been detected in zoonotic viruses in humans (Figure), PB1 in H10N8 and M in H7N9, but we did not find mutations known for mammalian host adaptation in these 2 segments. The encoded proteins of the PB1 and M gene segments that we isolated featured amino acid sequences rarely observed in mammalian and avian IAVs, including H9 (PB1, 97K, 156N, 397V, 535V, 688I, and 704T; M1, 31I and 46V; and M2, 25S). We could not determine whether these were random or adaptive mutations. The PB1 segment of avian H9N2 is highly compatible to other polymerase genes from mammalian IAVs (8). Such results suggest the need for further characterization of these mutations, particularly those in the PB1 gene.

A recent report in China discussed multiple Eurasian avian-like H1N1 swine influenza

reassortants with internal genes derived from pH1N1 and triple reassortant lineages (9). One group of these reassortants (genotype 4) displayed a genotype similar to A/swine/HK/NS419/2021, the only exception being that the virus's PB1 and M gene segments were of pH1N1 lineage. That report showed that genotype 4 Eurasian avian-like swine IAVs can bind to human sialic acid receptors (i.e., $\alpha 2,3$), enabling efficient virus replication in human airway epithelial cells, and achieve efficient aerosol transmission in ferrets (9). Serologic surveillance further showed that 10% of studied swine workers were positive for the genotype 4 reassortant (9). Our own sequence analyses suggest that some of the genotype 4 viruses and our Eurasian avian-like H1N1 viruses might share a common ancestry (e.g., A/swine/Shandong/1207/2016; Appendix Figures 1–6). Further risk assessment on the pandemic potential of this genotype and its reassortants is needed (10).

In summary, many zoonotic IAVs in humans have genes derived from H9N2 subtypes. Our results suggest that avian H9N2 IAVs are infecting swine and reassorting with swine IAVs, which indicates the need for continued monitoring of swine IAVs in both China and outlying regions.

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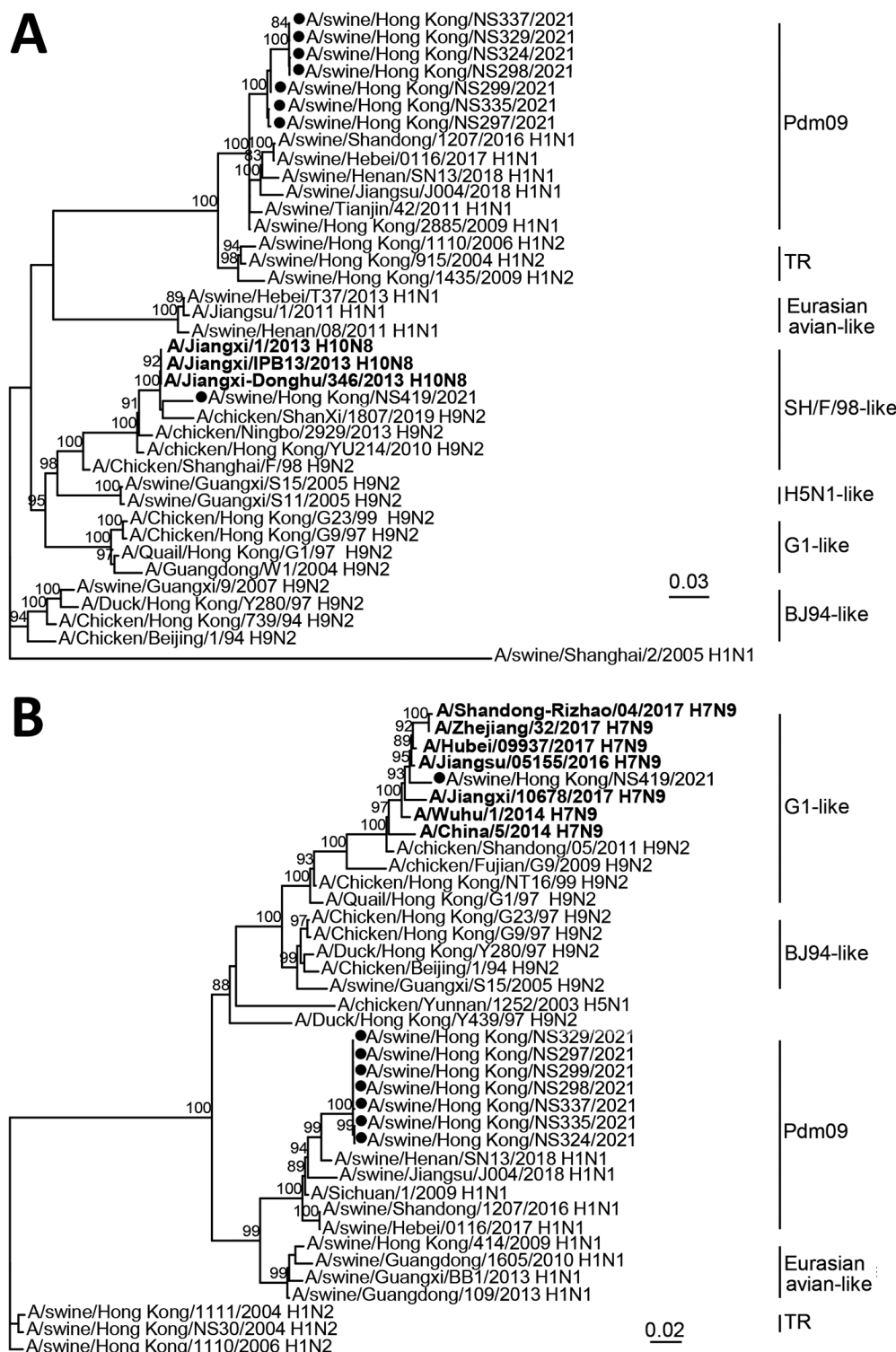


Figure. Phylogenetic tree of polymerase basic 1 (A) and matrix (B) gene sequences of swine influenza viruses from China and reference sequences. Bold indicates human H7N9 and H10N8 sequences. Viral sequences generated in this study (black circles) and those downloaded from public domains (Appendix Table, <https://wwwnc.cdc.gov/EID/article/28/7/22-0642-App1.pdf>) were aligned by using Muscle version 3.8 (<http://www.drive5.com/muscle>). Phylogenetic trees were constructed by IQ-TREE 1.6.12 (<http://www.iqtree.org>) by using the generalized time reversible plus gamma model. Major animal viral lineages are as shown. Bootstrap values $\geq 80\%$ are shown. Scale bar indicates estimated genetic distance.

About the Author

Miss Sun is a postgraduate student at The University of Hong Kong, Hong Kong, China. Her primary research interest is bioinformatics.

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Persistent SARS-CoV-2 Alpha Variant Infection in Immunosuppressed Patient, France, February 2022

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We describe persistent circulation of SARS-CoV-2 Alpha variant in an immunosuppressed patient in France during February 2022. The virus had a new pattern of mutation accumulation. The ongoing circulation of previous variants of concern could lead to reemergence of variants with the potential to propagate future waves of infection.

Immunosuppressed patients can have prolonged SARS-CoV-2 infection (1). Studies have reported the occurrence and selection of multiple mutations in the spike glycoprotein sequence in immunosuppressed patients with persistent SARS-CoV-2 infections (2–6). To date, intrahost mutations have been described essentially in the ancestral wild-type SARS-CoV-2 virus (3,5–8), especially during prolonged infection with variants of concern (VOCs) (9). Additional SARS-CoV-2 mutations in immunocompromised persons could enable increased virus transmissibility and immune evasion, shaping the emergence of new VOCs. We describe a new mutation accumulation pattern in SARS-CoV-2 Alpha virus in an immunosuppressed patient.

An 84-year-old woman with evolutive mantle cell lymphoma who was receiving maintenance rituximab and lenalidomide treatment was admitted to the hospital on May 17, 2021. She had asthenia, fever, and hypoxia (93% oxygen saturation). At admission (day 0), she tested positive for SARS-CoV-2 RNA (Figure). She had received 2 vaccine doses 84 and 66 days before admission. She did not have respiratory symptoms, but a chest computed tomography scan showed ground-glass opacities in her lungs. The patient was hospitalized and treated with corticosteroids for 10 days. She tested SARS-CoV-2-positive again on August 26, day 101 after her initial

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Appendix

Appendix Table. Viral sequences used in this study

Segment	Strain name	Accession number
PB2	A/swine/Fujian/204/2007	FJ536816
PB2	A/swine/Wisconsin/30954/1976	CY036806
PB2	A/swine/Shanghai/2/2005	FJ789824
PB2	A/swine/Hong Kong/915/2004	GQ229273
PB2	A/swine/Hong Kong/78/2003	GQ229313
PB2	A/swine/Hong Kong/1562/2005	GQ229337
PB2	A/swine/Hong Kong/1110/2006	GQ229377
PB2	A/swine/Hong Kong/1435/2009	CY061650
PB2	A/swine/Hong Kong/NS1583/2009	CY061722
PB2	A/swine/Hong Kong/2299/2009	CY061730
PB2	A/swine/Hong Kong/2314/2009	CY061738
PB2	A/swine/Hong Kong/NS1809/2009	CY061746
PB2	A/swine/Hong Kong/NS1810/2009	CY061754
PB2	A/swine/Hong Kong/2885/2009	CY061762
PB2	A/swine/Hong Kong/2886/2009	CY061770
PB2	A/swine/Hong Kong/2974/2009	CY061786
PB2	A/swine/Hong Kong/189/2010	CY061802
PB2	A/swine/Hong Kong/1795/1994	CY085102
PB2	A/swine/Hong Kong/8278/2001	CY085371
PB2	A/swine/Hong Kong/227/2002	CY085491
PB2	A/swine/Hong Kong/1248/2002	CY085523
PB2	A/swine/Hong Kong/1578/2003	CY085603
PB2	A/swine/Hong Kong/1111/2004	CY085659
PB2	A/swine/Hong Kong/729/2005	CY085731
PB2	A/swine/Hong Kong/72/2007	CY085811
PB2	A/swine/Hong Kong/NS252/2009	CY085995
PB2	A/swine/Guangdong/1605/2010	JN375017
PB2	A/swine/Guangdong/1623/2010	JN375023
PB2	A/swine/Shaanxi/s2/2012	JX963608
PB2	A/Jiangsu/1/2011	KF057091
PB2	A/swine/Guangdong/109/2013	KP404209
PB2	A/swine/Hunan/30/2013	KP404289
PB2	A/swine/Hong Kong/299/1993	CY084982
PB2	A/swine/Hong Kong/1223/1993	CY085054
PB2	A/swine/Hebei/0116/2017	MN416337
PB2	A/swine/Henan/SN13/2018	MN416362
PB2	A/swine/Jiangsu/J004/2018	MN416364
PB2	A/swine/Shandong/1207/2016	MN416383
PB1	A/Chicken/Hong Kong/G9/97	AF156416
PB1	A/Chicken/Hong Kong/G23/99	AF156417
PB1	A/Duck/Hong Kong/Y280/97	AF156419
PB1	A/Quail/Hong Kong/G1/97	AF156421
PB1	A/Chicken/Hong Kong/739/94	AF156422
PB1	A/Chicken/Beijing/1/94	AF156423
PB1*	A/Jiangxi-Donghu/346/2013	EPI_ISL_152846
PB1*	A/Jiangxi/1/2013	EPI_ISL_174265

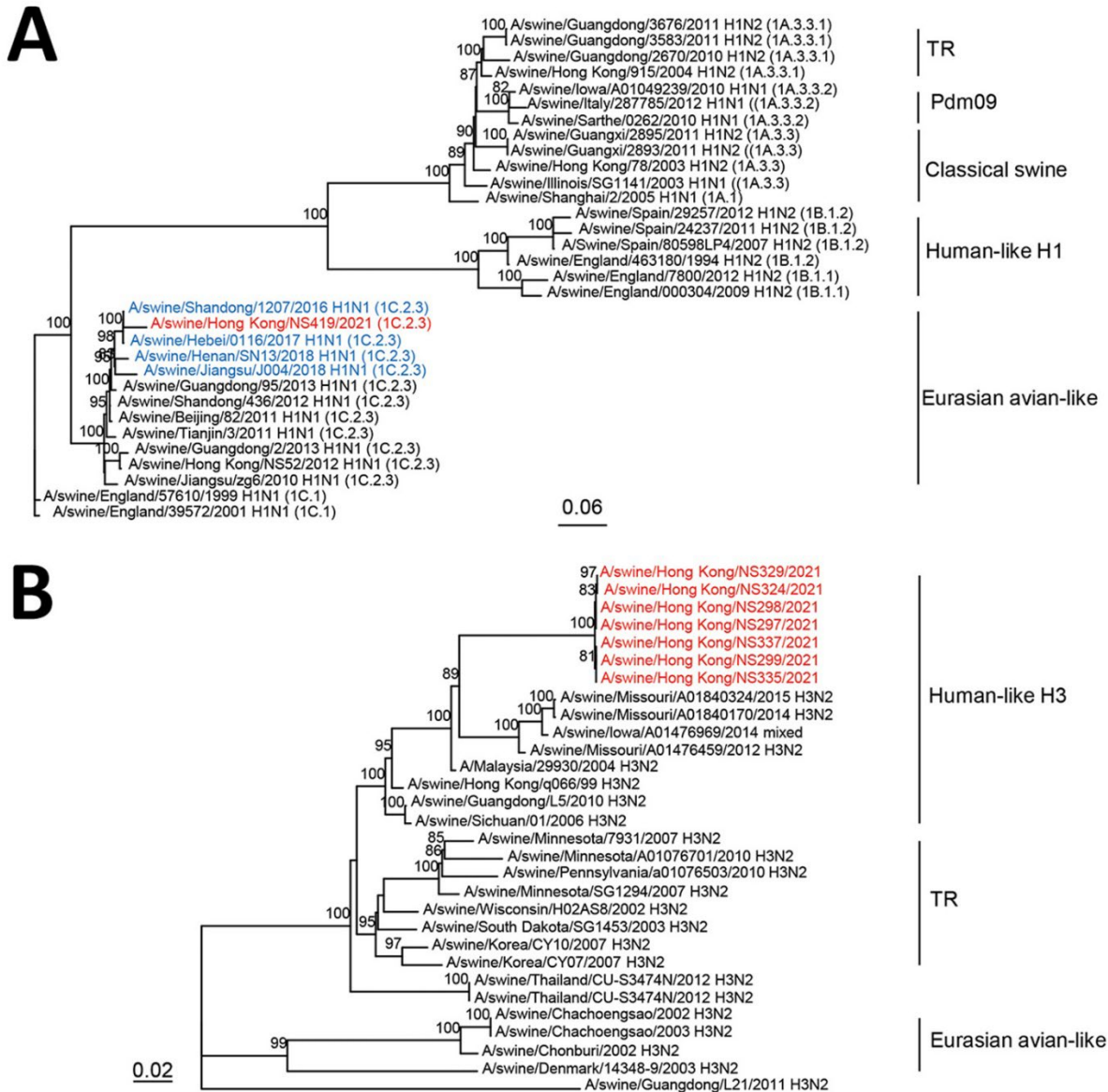
Segment	Strain name	Accession number
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PB1	A/swine/Hong Kong/915/2004	GQ229268
PB1	A/swine/Hong Kong/1110/2006	GQ229372
PB1	A/swine/Hong Kong/1435/2009	CY061651
PB1	A/swine/Hong Kong/2885/2009	CY061763
PB1	A/swine/Guangxi/9/2007	CY075044
PB1	A/Jiangsu/1/2011	KF057098
PB1	A/chicken/Hong Kong/YU214/2010	KF260616
PB1	A/Jiangxi/IPB13/2013	KJ406535
PB1	A/swine/Tianjin/42/2011	KP404402
PB1	A/chicken/Ningbo/2929/2013	KP415347
PB1	A/Guangdong/W1/2004	KX867847
PB1	A/swine/Hebei/0116/2017	MN416424
PB1	A/swine/Hebei/T37/2013	MN416433
PB1	A/swine/Henan/08/2011	MN416437
PB1	A/swine/Henan/SN13/2018	MN416449
PB1	A/swine/Shandong/1207/2016	MN416470
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PB1	A/swine/Jiangsu/J004/2018	MN416451
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PA	A/swine/Shanghai/2/2005	FJ789826
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PA	A/swine/Hong Kong/78/2003	GQ229312
PA	A/swine/Hong Kong/1562/2005	GQ229336
PA	A/swine/Hong Kong/1110/2006	GQ229376
PA	A/swine/Hong Kong/1435/2009	CY061652
PA	A/swine/Hong Kong/NS1583/2009	CY061724
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PA	A/swine/Hong Kong/NS1810/2009	CY061756
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PA	A/swine/Hong Kong/8278/2001	CY085373
PA	A/swine/Hong Kong/227/2002	CY085493
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PA	A/swine/Hong Kong/1578/2003	CY085605
PA	A/swine/Hong Kong/1111/2004	CY085661
PA	A/swine/Hong Kong/729/2005	CY085733
PA	A/swine/Hong Kong/NS252/2009	CY085997
PA	A/swine/Guangdong/1605/2010	JN375094
PA	A/swine/Guangdong/1623/2010	JN375099
PA	A/swine/Shaanxi/s2/2012	JX963609
PA	A/Jiangsu/1/2011	KF057105
PA	A/swine/Guangdong/109/2013	KP404211
PA	A/swine/Hunan/30/2013	KP404291
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HA	A/swine/England/39572/2001	CY116151
HA	A/swine/England/57610/1999	CY116222
HA	A/swine/Sarthe/0262/2010	FR871195
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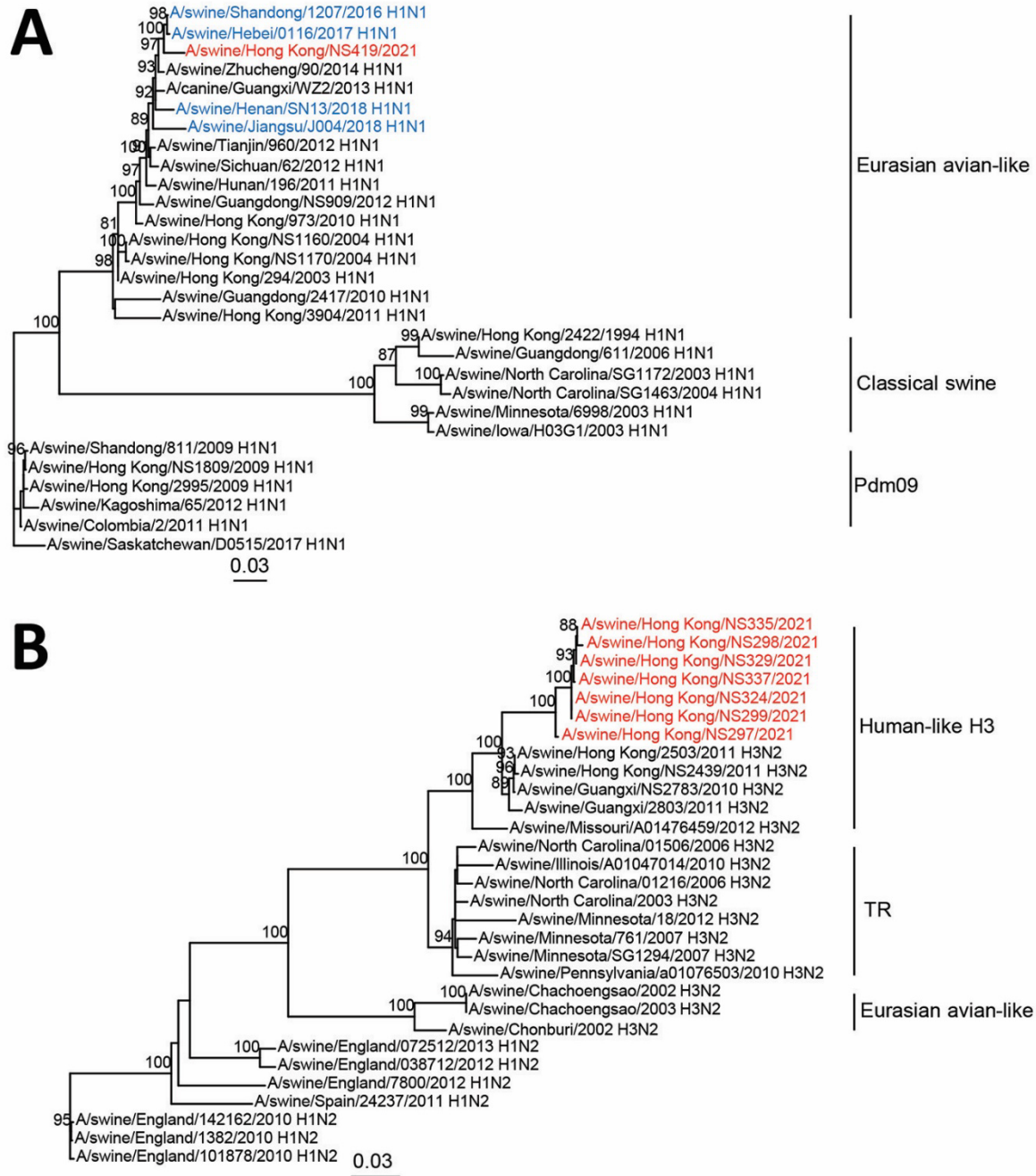
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NP	A/swine/Hong Kong/729/2005	CY085735
NP	A/swine/Hong Kong/72/2007	CY085815
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NA	A/swine/Minnesota/6998/2003	CY098486
NA	A/swine/Shandong/811/2009	JQ695889
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NA	A/swine/North Carolina/SG1463/2004	CY159195
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NA	A/swine/Sichuan/62/2012	KP404382
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NA	A/canine/Guangxi/WZ2/2013	MG254094
NA	A/swine/Saskatchewan/D0515/2017	MK462308
NA	A/swine/Tianjin/960/2012	MN416762
NA	A/swine/Chachoengsao/2003	AB434346
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NA	A/swine/Chonburi/2002	AB573015
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M*	A/Jiangxi/10678/2017_H7N9	EPI_ISL_285246
M*	A/Shandong-Rizhao/04/2017_H7N9	EPI_ISL_285268
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M*	A/Zhejiang/32/2017_H7N9	EPI_ISL_285320
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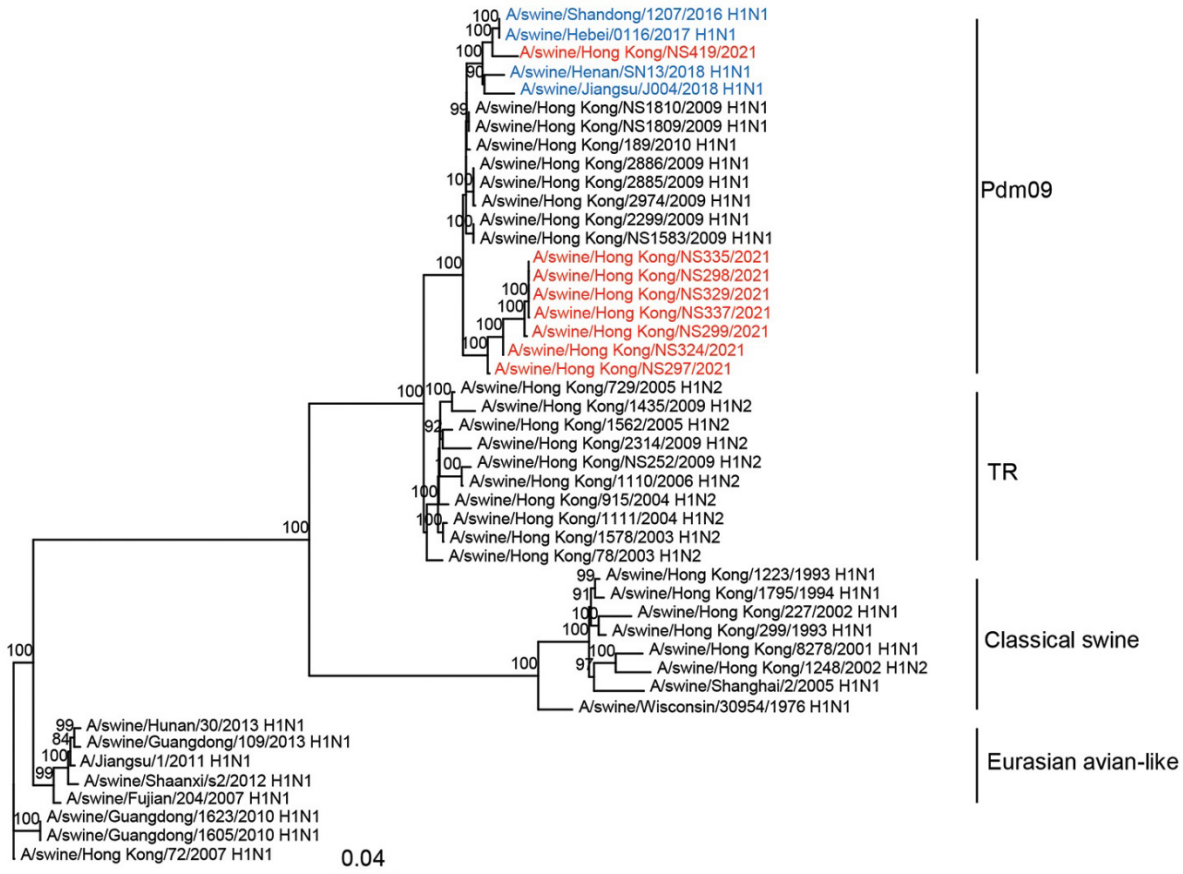
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NS	A/swine/Hong Kong/1578/2003	CY085610
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NS	A/swine/Hubei/01/2009	CY091741
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NS	A/swine/Guangdong/275/2010	JN375253
NS	A/swine/Guangdong/278/2010	JN375254
NS	A/swine/Guangdong/297/2010	JN375257
NS	A/swine/Guangdong/1437/2010	JN375267
NS	A/swine/Guangdong/1604/2010	JN375269
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NS	A/Jiangsu/1/2011	KF057140
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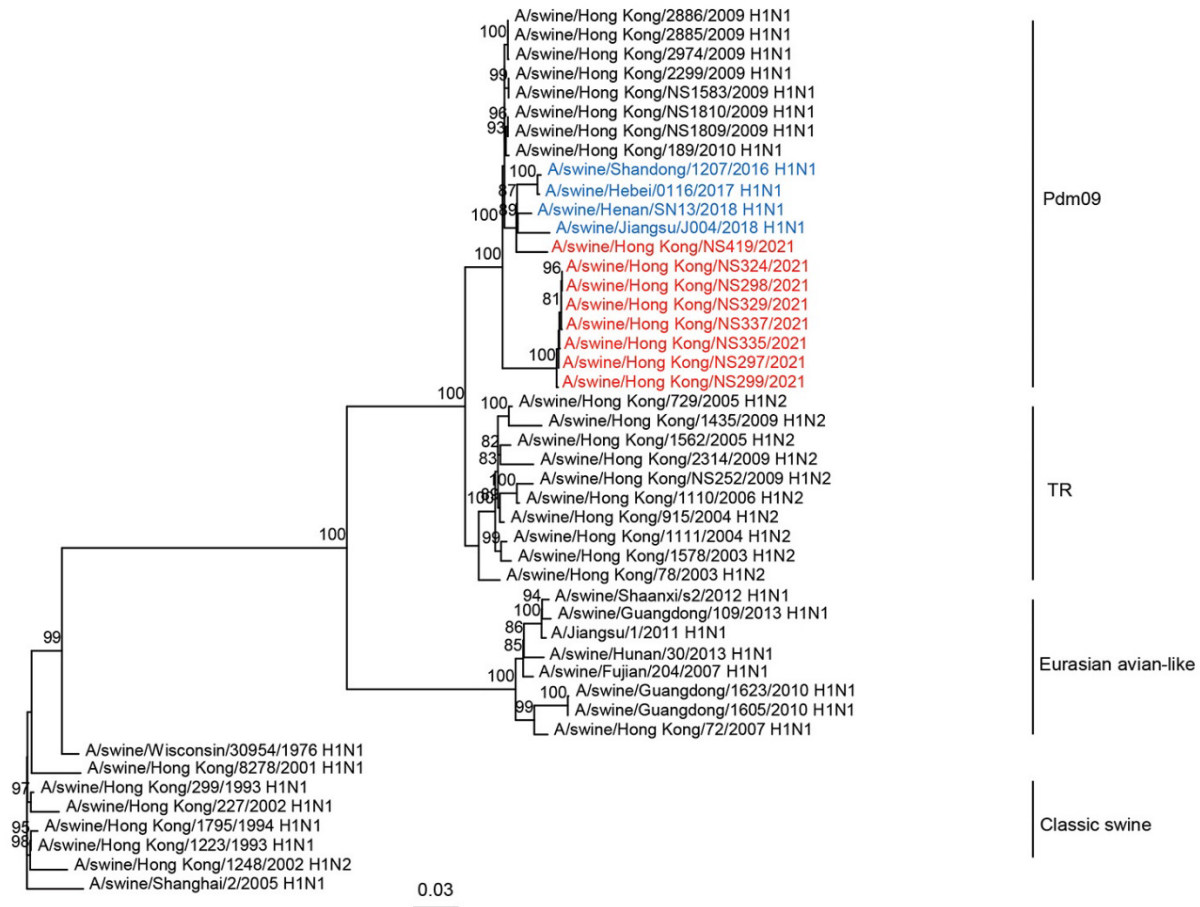
Appendix Figure 1. Phylogenetic tree of H1 (A) or H3 (B) gene sequences. Viral sequences generated from this study (red) and those downloaded from public domains (Appendix Table) were aligned using muscle v3.8 (<http://www.drive5.com/muscle/>). Virus sequences from G4 swine viruses (Main text) are highlighted in blue. Phylogenetic trees were then constructed by IQ-tree v1.6.12 (<http://www.iqtree.org/release/v1.6.12>) using the GTR+G model. Major animal viral lineages are as shown. The phylogenetic clades of the studied H1 sequences are shown in brackets (Anderson et al., mSphere 2016; <https://doi.org/10.1128/mSphere.00275-16>). Bootstrap values $\geq 80\%$ are shown. Scale bar indicates estimated genetic distance.



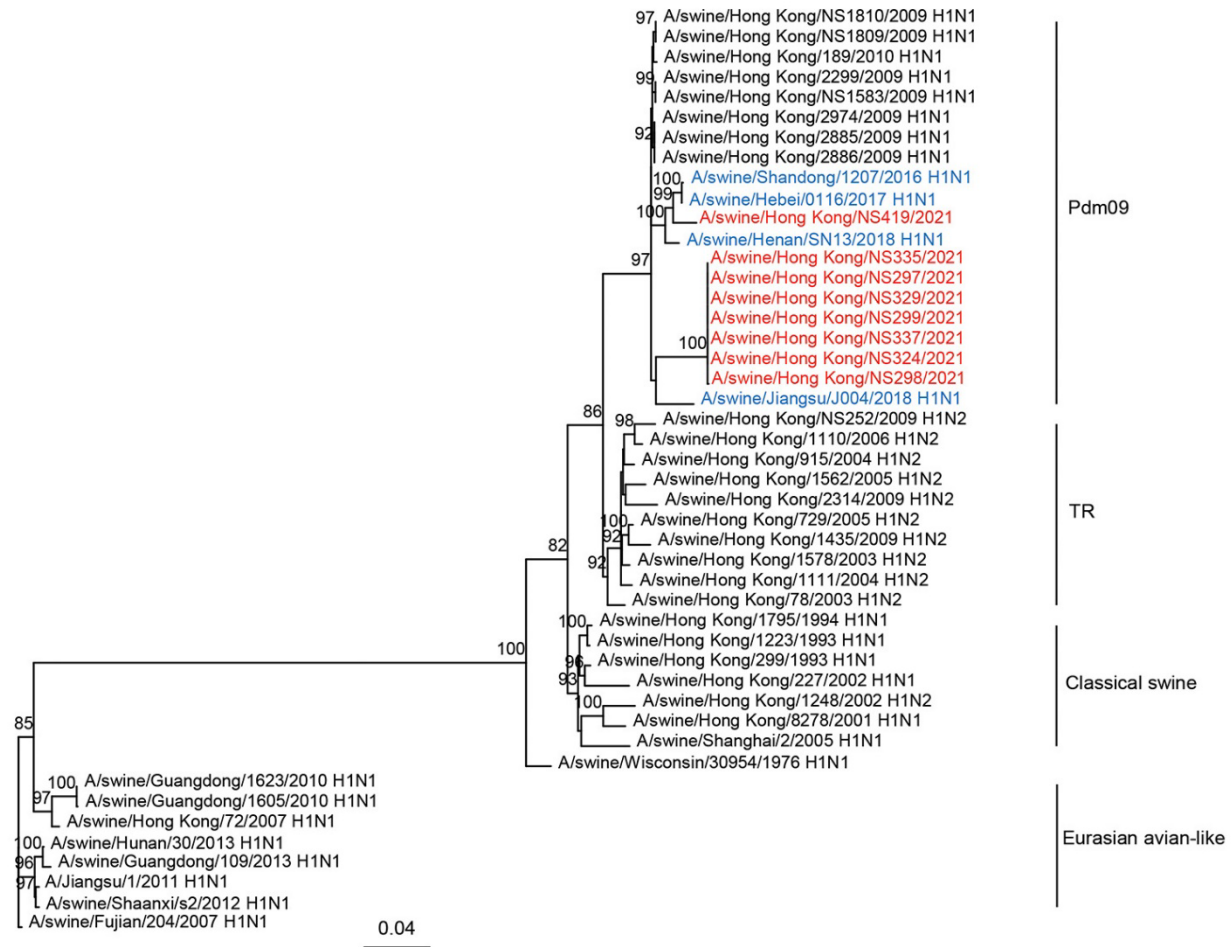
Appendix Figure 2. Phylogenetic tree of N1 (A) or N2 (B) gene sequences. Viral sequences generated from this study (red) and those downloaded from public domains (Appendix Table) were aligned using muscle v3.8 (<http://www.drive5.com/muscle/>). Virus sequences from G4 swine viruses (Main text) are highlighted in blue. Phylogenetic trees were then constructed by IQ-tree v1.6.12 (<http://www.iqtree.org/release/v1.6.12>) using the GTR+G model. Major animal viral lineages are as shown. Bootstrap values $\geq 80\%$ are shown. Scale bar indicates estimated genetic distance.



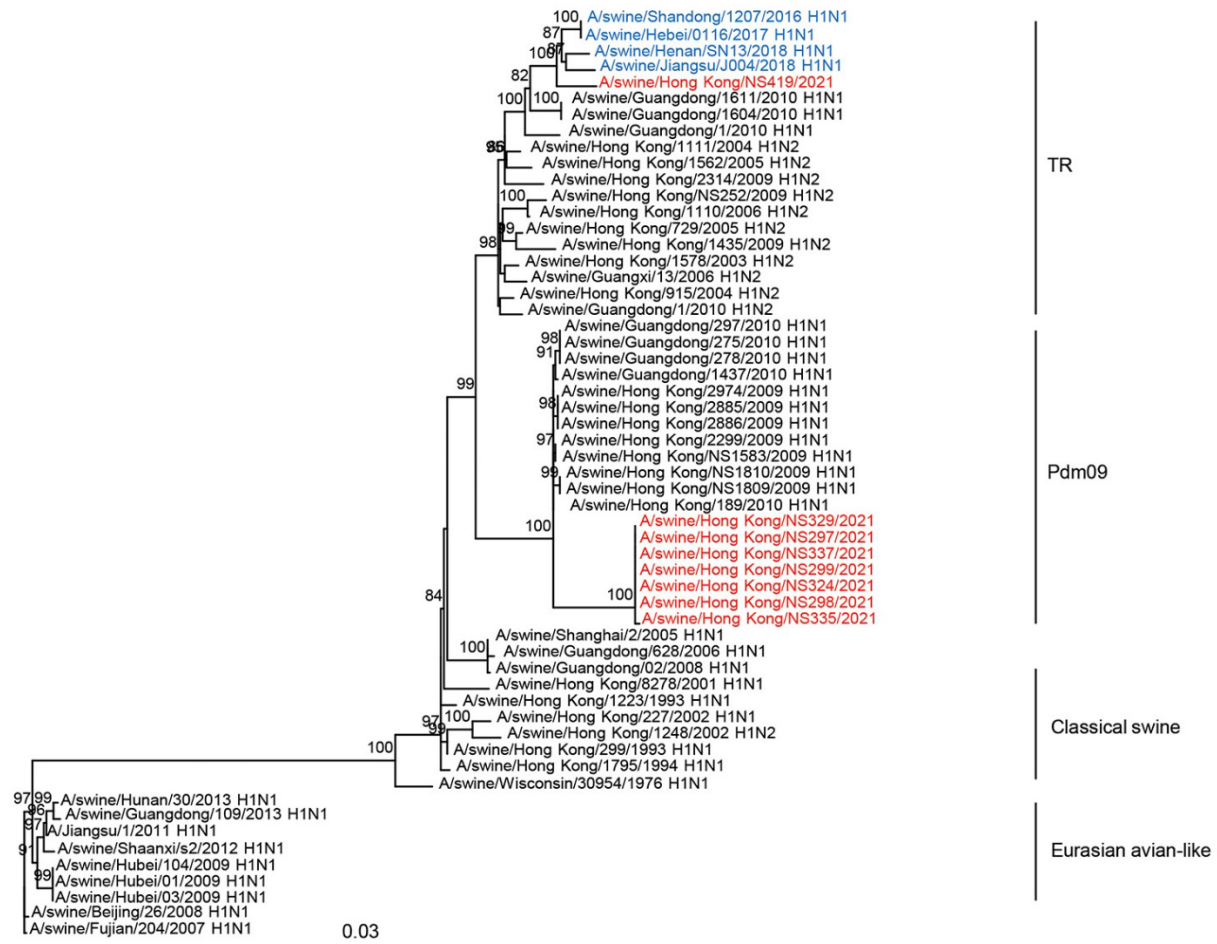
Appendix Figure 3. Phylogenetic tree of PB2 gene sequences. Viral sequences generated from this study (red) and those downloaded from public domains (Appendix Table) were aligned using muscle v3.8 (<http://www.drive5.com/muscle/>). Virus sequences from G4 swine viruses (Main text) are highlighted in blue. Phylogenetic trees were then constructed by IQ-tree v1.6.12 (<http://www.iqtree.org/release/v1.6.12>) using the GTR+G model. Major animal viral lineages are as shown. Bootstrap values ≥80% are shown. Scale bar indicates estimated genetic distance.



Appendix Figure 4. Phylogenetic tree of PA gene sequences. Viral sequences generated from this study (red) and those downloaded from public domains (Appendix Table) were aligned using muscle v3.8 (<http://www.drive5.com/muscle/>). Virus sequences from G4 swine viruses (Main text) are highlighted in blue. Phylogenetic trees were then constructed by IQ-tree v1.6.12 (<http://www.iqtree.org/release/v1.6.12>) using the GTR+G model. Major animal viral lineages are as shown. Bootstrap values $\geq 80\%$ are shown. Scale bar indicates estimated genetic distance.



Appendix Figure 5. Phylogenetic tree of M gene sequences. Viral sequences generated from this study (red) and those downloaded from public domains (Appendix Table) were aligned using muscle v3.8 (<http://www.drive5.com/muscle/>). Virus sequences from G4 swine viruses (Main text) are highlighted in blue. Phylogenetic trees were then constructed by IQ-tree v1.6.12 (<http://www.iqtree.org/release/v1.6.12>) using the GTR+G model. Major animal viral lineages are as shown. Bootstrap values $\geq 80\%$ are shown. Scale bar indicates estimated genetic distance.



Appendix Figure 6. Phylogenetic tree of NS gene sequences. Viral sequences generated from this study (red) and those downloaded from public domains (Appendix Table) were aligned using muscle v3.8 (<http://www.drive5.com/muscle/>). Virus sequences from G4 swine viruses (Main text) are highlighted in blue. Phylogenetic trees were then constructed by IQ-tree v1.6.12 (<http://www.iqtree.org/release/v1.6.12>) using the GTR+G model. Major animal viral lineages are as shown. Bootstrap values $\geq 80\%$ are shown. Scale bar indicates estimated genetic distance.